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Product Information

Protease Inhibitor Cocktail

For Bacterial Cells

Product Number **P 8465**

Storage Temperature -20°C

Product Description

Crude cell extracts contain a number of endogenous enzymes, such as proteases and phosphatases, which are capable of degrading the proteins present in the extract. The best way to improve the yield of intact proteins is to add inhibitors of these enzymes known to be present in the source material. This protease inhibitor cocktail has been optimized and tested for bacterial cell use.

This is a mixture of protease inhibitors with a broad specificity for the inhibition of serine, cysteine, aspartic, metalloproteases, and aminopeptidases.

Preparation Instructions

The cocktail solution may be prepared as follows:

To the 5 ml size, add 1 ml of dimethyl sulfoxide (DMSO), vortex for one minute, then add 4 ml of deionized water. The resulting solution should be clear.

To the 25 ml size, add 5 ml of DMSO, vortex for one minute, then add 20 ml of deionized water. The resulting solution should be clear.

If less than 5 ml or 25 ml of cocktail solution is required, dissolve 215 mg of the lyophilized powder per ml of DMSO and add 4 volumes of water.

Storage/Stability

The lyophilized powder is stable for at least 2 years when stored unopened at -20°C .

The prepared solution in DMSO and water will remain clear and colorless for approximately 24 hours at 4°C . However, after this time the inhibitors tend to precipitate from the DMSO and water solution. It is recommended to prepare solutions of this product fresh when needed.

Procedure

One ml of the cocktail solution is recommended for the inhibition of the protease activity found in 20 ml of cell lysate from 4 g (wet weight) of *E. coli* cells. The *E. coli* cells were grown on either BHI or NZ-Amine B. Since not all organisms contain the same level of endogenous proteases, it may sometimes be necessary to increase the concentration of inhibitors.

Components

The reconstituted cocktail solution contains the following concentrations of inhibitors:

AEBSF	23 millimolar
EDTA	100 millimolar
Bestatin	2 millimolar
Pepstatin A	0.3 millimolar
E-64	0.3 millimolar

Product Profile

The individual components of this protease inhibitor cocktail have specific inhibitory properties. A description of each inhibitor is given below.

AEBSF (Product Code A 8456) inhibits serine proteases, such as trypsin and chymotrypsin.

EDTA (Product Code E 1644) inhibits metalloproteases.

Bestatin (Product Code B 8385) inhibits aminopeptidases, such as leucine aminopeptidase and alanyl aminopeptidase.^{1,2,3,4}

Pepstatin A (Product Code P 4265) inhibits acid proteases, such as pepsin (human or porcine), renin, cathepsin D, chymosin (bovine rennin), and protease B (*Aspergillus niger*).

E-64 (Product Code E 3132) inhibits cysteine proteases, such as calpain, papain, cathepsin B, and cathepsin L.

References:

1. Umezawa H., Ann. Rev. Microbiol., **36**, 75-99 (1982).
2. Aoyagi, T. et al, Biochem. Int., **9**, 405-411 (1984).
3. Aoyagi, T., & Umezawa, H., Acta Biol. Med. Ger., **40**, 1523-1529 (1981).
4. Mumford, R. A., et al, Biochem. Biophys. Res. Comm., **103**, 565-572 (1981).

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